(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/68597 A1

(51) International Patent Classification[7]: C07C 323/41, 323/44, A61K 31/165, 31/17, A61P 35/00, 19/02

(21) International Application Number: PCT/EP01/02739

(22) International Filing Date: 12 March 2001 (12.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2000A000554 17 March 2000 (17.03.2000) IT

(71) Applicant *(for all designated States except US)*: **INDENA S.P.A.** [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOMBARDELLI, Ezio** [IT/IT]; Via Val di Sole, 22, I-20141 Milano (IT). **PONTIROLI, Alessandro** [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

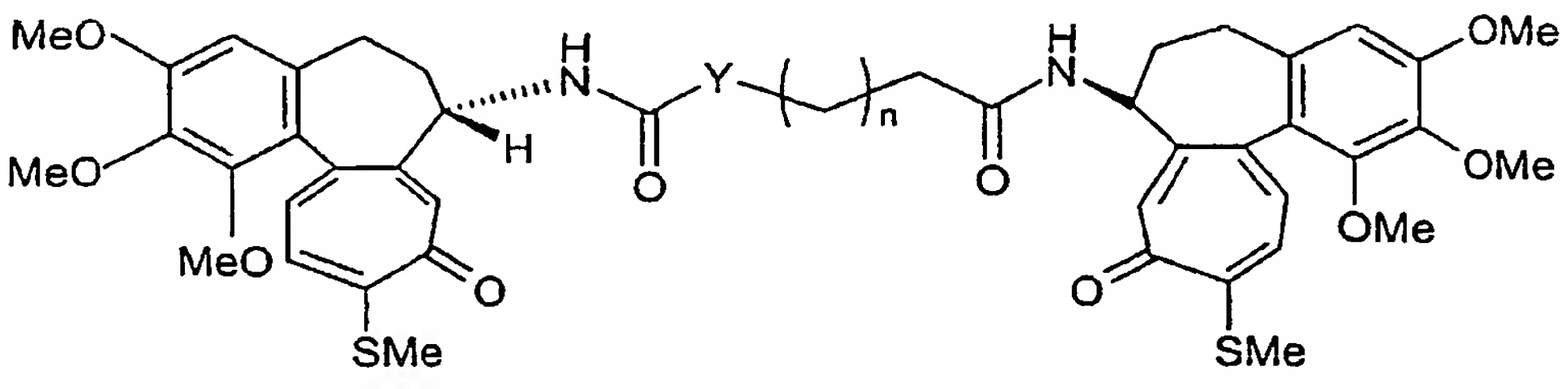
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/68597 A1

(54) Title: N-DEACETYLTHIOCOLCHICINE DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

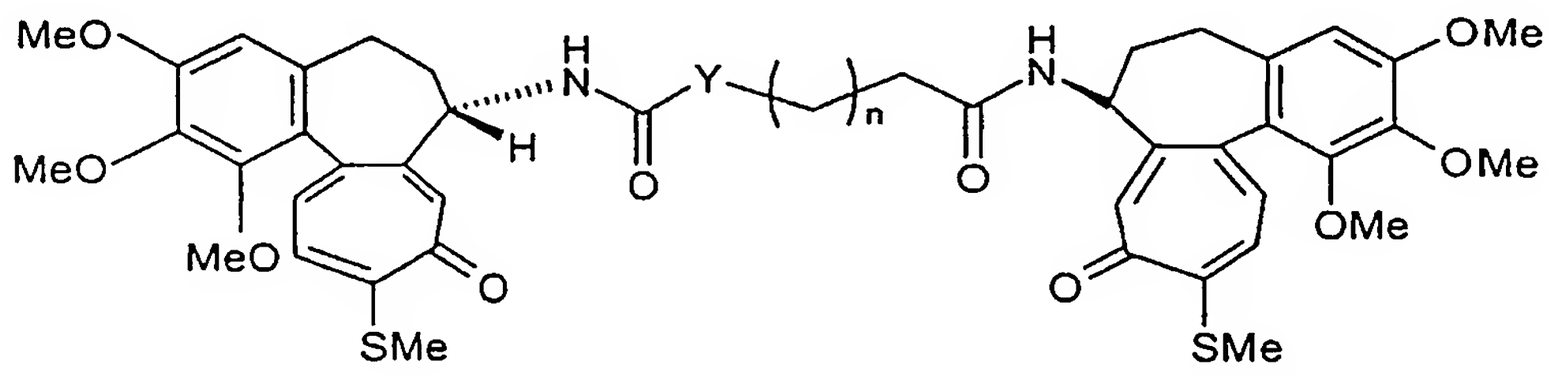


(I)

(57) Abstract: Derivatives of N-deacetyl- thiocolchicine or of the isoster thereof of formula (I), wherein: n is an integer of 0 to 8; Y is a CH_2 group or, when n is 1, can also be a group of formula NH. Compounds (I) have anti-proliferative activity.

N-DEACETYLTHIOCOLCHICINE DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

The present invention relates to derivatives of N-deacetyl-thiocolchicine or of the isoster thereof of formula (I)



(I)

wherein:

n is an integer of 0 to 8;

Y is a CH_2 group or, when n is 1, can also be a group of formula NH.

Colchicines and thiocolchicines are known antiblastic compounds capable of destabilizing microtubules through interaction with tubulin.

Colchicine is currently used in the therapy of gout and related inflammatory conditions, but its use is restricted to the acute phases due to its high gastro-intestinal toxicity.

A number of colchicine or thiocolchicine derivatives have been studied, in view of a possible use thereof as antitumor medicaments, but the efforts of researchers have to date been unsuccessful due to the often very restricted therapeutical index of such compounds.

Only one colchicine derivative, demecolcine, has been used in the past in clinic for the treatment of leukemias, but with poor success.

It has now been found that the compounds of formula (I) have anti-proliferative activity, in particular on cells expressing the MDR (multi-drug

resistance) phenotype, with an approximately 1:1 ratio of activity on sensitive cells to activity on resistant cells.

The compounds of the invention have in fact powerful antimitotic activity and are characterized by favorable therapeutic index which makes them suitable for the therapeutical treatment of various forms of tumors, as well as for degenerative rheumatoid arthritis, a disease characterized by excessive proliferation and abnormal migration of leukocytes.

Compounds (I) have cytotoxicity comparable to that of the most effective antitumor medicaments, while having a remarkably wider action spectrum, particularly against cells resistant to known drugs.

Compounds (I) wherein Y is CH_2 are prepared by reacting N-deacetyl-thiocolchicine with dicarboxylic acid reactive derivatives in dry solvents. Examples of suitable dicarboxylic acid reactive derivatives comprise chlorides, reactive anhydrides or esters, in particular N-hydroxysuccinyl diesters obtainable by reacting said acids with N-hydroxy-succinimide. The reaction is preferably carried out in solvents such as ethyl ether, dioxane or tetrahydrofuran in the presence of bases, for example triethylamine.

On the other hand, compounds (I) wherein Y is NH and n is 1 can be prepared by reacting N-deacetyl-thiocolchicine with N-hydroxy-succinimide in the presence of amines and condensing agents such as dicyclohexylcarbodiimide (DCC), in a suitable aprotic solvent, preferably a chlorinated hydrocarbon (methylene chloride, chloroform). Said compounds can also be obtained as side-products from the reaction between dicarboxylic acid N-hydroxysuccinyldiesters and N-deacetyl-thiocolchicine.

The activity of these compounds was evaluated on a wide number of resistant tumour cells expressing the MDR phenotype; these compounds proved to be particularly active on different sensitive colon lines expressing MDR.

The following Table reports by way of example the activity of these two

compounds, comparing their biological activity to thiocolchicine and taxol as reference molecules.

TABLE

Compounds	IC_{50} nM		
	MCF7	MCF7-ADRr	MCF7-ADRr/MCF7
Tiocol 39 (Ex. 1)	12	43	3.58
Tiocol 43 (Ex. 4)	21	36	1.71
Tiocol 54 (Ex. 2)	2.6	2.8	1.07
Thiocolchicine	0.02	400	20000

The cytotoxic activity was evaluated according to M.C. Alley et al., *Cancer Research, 48, 589-601, 1998.*

The above-reported data evidence the high cytotoxic activity of the compounds of the invention on both sensitive cell lines and different drug-resistant cell lines to various antitumor drugs.

The compounds of the invention are therefore useful in the treatment of proliferative pathologies and in particular tumors of various origins, rheumatoid arthritis or other degenerative pathologies wherein antiproliferative and anti-inflammatory actions are indicated.

For this purpose, compounds (I) will be administered in the form of pharmaceutical compositions suitable to the oral, parenteral, epicutaneous or transdermal administrations. The dosage of compounds (I) will range from 1 to 100 mg/m^2 body area, depending on the administration route. The compounds will preferably be administered orally.

Examples of compositions comprise capsules, tablets, vials, creams, solutions, granulates.

The following examples illustrate the invention in greater detail.

EXAMPLE 1

Preparation of compound (I) wherein Y is CH_2 and n is 2 (Tiocol 39)

100 mg of N-deacetyl-thiocolchicine (M.W. = 373 g/mol, 0.27 mmol) are dissolved in 6 ml of dry dioxane at room temperature under nitrogen atmosphere. 46 mg of adipic acid activated as N-hydroxy succinyl diester (M.W. = 340 g/mol, 0.135 mmol) and 40 µl of dry triethylamine (M.W. = 101 g/mol, d=0.726 g/ml, 0.27 mmol) are added. The mixture is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: $CHCl_3$: MeOH = 95:5). The solvent is evaporated off and the product is recovered by flash chromatography on silica (eluent: $CHCl_3$: MeOH = 75:1).

Yield: 85%

EXAMPLE 2

Preparation of the compound (I) wherein Y is NH n is 1 (Tiocol 54)

A solution of 1 g of deacetyl-thiocolchicine in 40 ml of dry CH_2Cl_2 is added with 154 mg of N-hydroxysuccinimide, 276 mg of DCC and 476 µl of diisopropylethylamine. The mixture is refluxed under nitrogen for at least 2 days. The progress of the reaction is monitored by TLC (CH_2Cl_2-EtOH=95/5). The mixture is concentrated to small volume and the residue is taken up with ethyl acetate. The product is left to crystallize, then further purified by flash chromatography (eluent AcOEt - hexane 7/3 or (CH_2Cl_2-EtOH=95/5). 500 mg of product are obtained.

^{1}H-NMR(DMSO-d6-300Mhz): 8.80 d; 7.82 br s; 7.75-7.60 S; 7.37;7.18; 6.59; 4.90 m; 4.66 m; 4.52 dd; 3.96 s ppm.

^{13}C-NMR($CDCl_3$): 182.5; 181.9; 172.2; 158.0; 175.5; 157.1; 153.8; 153.7; 153; 152.3; 151.3; 151.2; 141.6; 141.5; 139.4; 139.3; 135.5; 135.5 d, 134.8; 134.7; 129.0; 128.4 (d).

MS(m/z) 866.4 [(M+Na)+].

EXAMPLE 3

Preparation of compound (I) wherein Y is CH_2 and n is 6 (Tiocol 33)

200 mg of N-deacetyl-thiocolchicine (M.W. = 373 g/mol, 0.54 mmol) are dissolved in 12 ml of dry dioxane at room temperature under nitrogen atmosphere. 91.8 mg of sebacic acid activated as N-hydroxy succinyl diester (M.W. = 396 g/mol, 0.27 mmol) and 75 µl of dry triethylamine (M.W. = 101 g/mol, d= 0.726 g/ml, 0.54 mmol) are added. The mixture is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: $CHCl_3$:MeOH = 95:5), then after 20 hours is heated to 50°C and the solvent is evaporated off. The reaction crude is purified by flash chromatography on silica (eluent: $CHCl_3$:MeOH=40:1), to obtain 30 mg of a mixture of the title compound (with Rf:=0.3) and of the compound of example 2.

EXAMPLE 4

Preparation of compound (I) wherein Y is CH_2 and n is 0 (Tiocol 43)

Procedure A

190 mg of N-deacetyl-thiocolchicine (M.W.=373 g/mol, 0.512 mmol) are dissolved in 6 ml of dry dioxane at room temperature under nitrogen atmosphere. 80 mg of succinic acid activated as N-hydroxy succinyl diester (M.W. = 312 g/mol, 0.256 mmol) and 70 µl of dry triethylamine (M.W. = 101 g/mol, d=0.726 g/ml, 0.512 mmol) are added. The mixture is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: $CHCl_3$: MeOH = 95:5). The solvent is evaporated off, the residue is taken up with AcOEt to remove the residual N-deacetyl thiocolchicine and triethylamine (the product is insoluble).

Yield: 45%

Procedure B

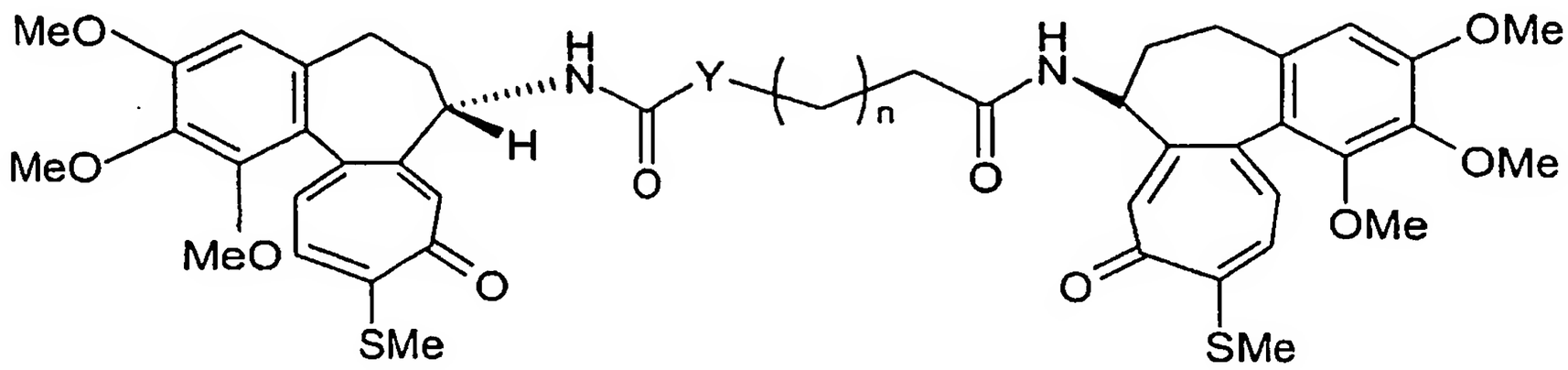
100 mg of N-deacetyl-N-succinyl-thiocolchicine (M.W.=473 g/mol, 0.21 mmol) are dissolved in 8 ml of dry CH_2Cl_2 at room temperature under nitrogen atmosphere. 93 mg of BOP (M.W. = 442,3 g/mol, 0.21 mmol) and 60 µl of dry

triethylamine (M.W. = 101 g/mol, d=0.726 g/ml, 0.42 mmol) are added. After 10 minutes, 80 mg of N-deacetyl thiocolchicine (M.W. = 101 g/mol, d=0.726 g/ml, 0.42 mmol) are added to the mixture, which is stirred at room temperature under nitrogen atmosphere for 48 hours (TLC control: $CHCl_3$: MeOH = 95:5). The solvent is evaporated off and the residue is taken up with AcOEt to remove the residual N-deacetyl thiocolchicine and triethylamine (the product is insoluble).

Yield: 45%

CLAIMS

1. Compounds of formula (I)



(I)

wherein:

n is an integer of 0 to 8;

Y is a CH_2 group or, when n is 1, can also be a group of formula NH.

2. Compounds as claimed in claim 1 wherein Y is CH_2.

3. Compositions as claimed in claim 1 wherein Y is NH.

4. Pharmaceutical compositions containing a compound of claims 1-3.

5. The use of the compounds of claims 1-3 for the preparation of medicaments for the treatment of tumors and rheumatoid arthritis.

INTERNATIONAL SEARCH REPORT

Interm al Application No
PCT/EP 01/02739

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C323/41 C07C323/44 A61K31/165 A61K31/17 A61P35/00
A61P19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BEILSTEIN Data, WPI Data, PAJ, EPO-Internal, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M.L. GELMI, ET AL.: "N-Deacetyl-N-aminoacylcolchicine derivatives: synthesis and biologial evaluation on MDR-negative human cancer cell lines" JOURNAL OF MEDICINAL CHEMISTRY, vol. 42, no. 25, 25 November 1999 (1999-11-25), pages 5272-5276, XP002170300 American Chemical Society, Washington, DC, US ISSN: 0022-2623 the whole document ---	1-5
A	WO 97 01570 A (INDENA) 16 January 1997 (1997-01-16) the whole document ---	1-5

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2001	05/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	English, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 01/02739

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	Q. SHI, ET AL.: "Antitumour agents. 172. Synthesis and biological evaluation of novel deacetamidothiocolchicin-7-ols and ester analogues as antitubulin agents." JOURNAL OF MEDICINAL CHEMISTRY, vol. 40, no. 6, 14 March 1997 (1997-03-14), pages 961-966, XP002038714 American Chemical Society, Washington, DC, US ISSN: 0022-2623 the whole document ---	1-5
A	US 4 533 675 A (A. BROSSI, ET AL.) 6 August 1985 (1985-08-06) the whole document ---	1-5
A	WO 97 47577 A (INDENA) 18 December 1997 (1997-12-18) the whole document -----	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
PCT/EP 01/02739

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9701570 A	16-01-1997	IT MI951367 A	27-12-1996
		AU 717082 B	16-03-2000
		AU 6414696 A	30-01-1997
		CA 2225284 A	16-01-1997
		EP 0840738 A	13-05-1998
		HU 9901685 A	28-09-1999
		JP 11501324 T	02-02-1999
		JP 3130050 B	31-01-2001
		NO 976001 A	19-12-1997
		PL 324621 A	08-06-1998
		US 5880160 A	09-03-1999
US 4533675 A	06-08-1985	NONE	
WO 9747577 A	18-12-1997	IT MI961168 A	09-12-1997
		AU 723752 B	07-09-2000
		AU 3027897 A	07-01-1998
		CA 2252717 A	18-12-1997
		CN 1219924 A	16-06-1999
		CZ 9804010 A	12-05-1999
		EP 0906262 A	07-04-1999
		HU 0001827 A	28-05-2001
		JP 2000503669 T	28-03-2000
		NO 985676 A	04-12-1998
		PL 330284 A	10-05-1999
		SK 167998 A	12-07-1999
		US 6080739 A	27-06-2000